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Optimization of Ultrasound-Assisted Extraction of Carotenoids from Ripe Papaya (*Carica papaya*) Powder: Kinetic Yield and Bioactive Profile Characterization

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Abstract
This study optimized the processing configurations for ultrasound-assisted extraction (UAE) to isolate bioactive carotenoids and structural polyphenols from dried ripe papaya (*Carica papaya*) powder using an aqueous ethanol system. The independent processing parameters were systematically monitored across three extraction temperatures (45°C, 55°C, and 65°C) and four discrete contact intervals (15, 20, 25, and 30 minutes). The optimal operating benchmark was established at 55°C for a processing duration of 25 minutes, achieving a maximum gravimetric extraction yield of 87.50 ± 0.02%. Detailed biochemical profiling of the pure extract under these optimized parameters revealed high baseline densities of target lipophilic pigments and functional bioactive components: a β-carotene content of 85.315 ± 0.02 mg/100g, a lycopene content of 55.568 ± 0.02 mg/100g, a total phenolic content (TPC) of 205.70 ± 0.03 mg/100g, and an overall free-radical scavenging capacity of 25.00 ± 0.02%. The empirical evidence confirms that localized acoustic cavitation effectively disrupts the rigid matrix framework of the fruit powder, introducing a highly efficient, ecologically sustainable, and clean-label processing technique for generating high-value functional botanical ingredients tailored for modern food applications.

Keywords: *Carica papaya*, Ultrasound-assisted extraction, β-carotene, Lycopene, Antioxidant activity, Kinetic yield

Introduction
In modern industrial food processing, natural pigments and clean-label functional additives have shifted from simple mark*et al*ternatives to core formulation requirements. Traditional synthetic colorants—specifically azo-linked coal tar derivatives such as Sunset Yellow FCF—face increasing regulatory and consumer scrutiny due to their reported associations with allergenicity, hypersensitivity, and behavioral updates in sensitive demographics. This shift has driven commercial processors to locate botanical sources that function as dual-action natural pigments and functional protective antioxidants.

Papaya (*Carica papaya*), an economically vital member of the Caricaceae family, represents an excellent plant matrix for target extraction. It naturally accumulates high concentrations of health-promoting phytochemicals, including core carotenoid fractions, ascorbic acid, complex flavonoids, and soluble dietary fibers. Carotenoids themselves are valuable lipophilic tetraterpenoid structures composed of repeating isoprene building blocks. Because the human metabolic system lacks the biochemical pathways required to synthesize these lipophilic molecules endogenously, they must be acquired through structured dietary intake. Regular consumption provides vital health advantages, including pro-vitamin A bio-conversion, heightened immunological defense responses, and a reduced incidence of age-related macular degradation. In ripe orange and red papaya tissues, this pigment profile is dominated by lipophilic fractions of β-carotene, lycopene, and β-cryptoxanthin. While lycopene works efficiently as a singlet-oxygen quencher, β-carotene possesses symmetric β-ionone ring structures that function as direct precursors for systemic retinal biogenesis within mammalian mucosal linings.

Extracting these valuable lipophilic solutes via conventional solid-liquid techniques—such as long maceration, mechanical magnetic stirring, or direct boiling—presents significant process limitations. These older techniques typically require extensive processing durations, consume large amounts of organic solvents, and deliver poor target recovery efficiency. Furthermore, exposing highly unsaturated carotenoid double-bond lines to long thermal processing accelerates trans-to-cis structural isomerization and auto-oxidation, degrading their functional and color-imparting qualities. To address these recovery challenges, advanced non-thermal extraction technologies are being integrated into manufacturing lines. Ultrasound-assisted extraction (UAE) utilizes the physical energy of acoustic cavitation. As high-frequency ultrasonic waves propagate through a liquid medium, they generate alternating cycles of compression and rarefaction. These pressure differentials create microscopic cavitation bubbles that grow until they reach an unstable size and violently implode near the surface of the plant tissue. The resulting localized micro-jets and high-pressure shear fields disrupt cell wall matrix polymers, reducing particle dimensions and accelerating the mass transfer of internal solutes into the solvent phase without requiring high, destructive thermal inputs.

While various studies have evaluated the extraction of generic polyphenols from fruit processing processing waste, there remains a lack of specific empirical modeling regarding the interactive behavior of extraction temperature and acoustic duration on the simultaneous recovery of isolated β-carotene, lycopene, and total free-radical scavenging components from pre-dried, concentrated ripe papaya powder. Therefore, this investigation models the exact process parameter kinetics (45–65°C; 15–30 min) of UAE to determine the optimal processing framework for maximizing total gravimetric mass yield alongside its associated bioactive profile.

Materials and Methods

Reagents and Source Material

Commercial-grade pre-dried ripe papaya (*Carica papaya*) powder was obtained from Naturall Annapurna, Agro Export (Bharadpur, Manawar Dhar, Madhya Pradesh, India) and preserved within light-shielded, vacuum-sealed desiccators to prevent moisture uptake and photo-oxidation. The analytical-grade chemical solvents and reagents used—including absolute ethanol (60%), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3) ,2,2-diphenyl-1-picrylhydrazyl (DPPH) radical crystals, water-saturated n-butanol, acetone, and hexane—were acquired from Merck and Sigma-Aldrich.

Ultrasound-Assisted Extraction (UAE) Process Formulation

The isolation protocol was adapted from standard solid-liquid extraction methodologies with specific configurations designed to monitor process kinetics. For each individual processing replication, a 2 g sample of ripe papaya powder was suspended in 100 mL of a 60% aqueous ethanol solvent matrix to maintain a strict, standardized solid-to-solvent load ratio of 1:50. The resulting slurry was transferred into a digital probe ultrasonication chamber operating at a fixed frequency of 40 kHz with an applied power output of 150W.

The independent processing parameters were systematically evaluated across a multi-variable testing matrix:

- **Sonication Temperature Level:** 45°C, 55°C, and 65°C
- **Acoustic Contact Duration:** 15, 20, 25, and 30 minutes

Following the completion of the designated sonication run, the raw liquid matrix was clarified by filtration through Whatman Filter Paper No. 1. The collected solvent filtrates were transferred to a digital water bath maintained between 60–80°C for 2–3 hours to drive off solvent fractions and isolate the target compounds. The remaining thick, solvent-free fluid extract concentrates were allowed to equilibrate to room temperature and stored in light-exclusion amber glass vessels at 4°C prior to chemical analysis.

Gravimetric Extraction Yield Determination

The total soluble mass recovery efficiency achieved by the process was calculated gravimetrically using the structural relationship outlined by Sharma *et al*. (2016) [15]:

$$\textbf{Extraction Yield (\%)} = (W_r / W_p) \times 100$$

Where:

- W_r = net dry weight of the extract residue obtained after solvent evaporation (g)
- W_p = initial dry weight of papaya powder used for extraction (2 g)

Biochemical Profile Characterization

Spectrophotometric Quantification of β-Carotene

The total accumulated β-carotene content within the isolated extract was quantified using the methodology described by Pandey *et al*. (2015) [14]. The optical density of the solution phase was recorded at 440 nm using a double-beam UV-Vis spectrophotometer. Pure water-saturated butanol (WSB)—formulated by mixing n-butanol and distilled water in an 8:2 volume ratio—was used as the calibration blank. The absolute quantitative concentration was calculated using the following equation:

$$C = (A_x \times C_s \times V) / (A_s \times P)$$

Where:

- C = total carotenoid concentration (µg/g)
- A_x = absorbance of the extract sample measured at 440 nm
- C_s = concentration of the reference standard (µg/mL)
- V = total dilution volume of the extract solution (mL)
- A_s = absorbance of the reference standard
- P = weight of the sample used for analysis (g)

Spectrophotometric Quantification of Lycopene

The baseline lycopene fractions were isolated and measured according to the parameters established by Fish *et al*. (2002) [10]. Duplicate extract portions (0.6 g) were weighed directly into 40 mL amber screw-cap glass vials containing a mixture of 5 mL of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 mL of 95% USP-grade ethanol, and 10 mL of pure hexane.

The extraction bottles were placed on a digital orbital shaker operating at 180 RPM for 15 minutes over an ice bath to prevent thermal degradation. Following extraction, 3 mL of chilled deionized water was added to induce distinct phase separation, and the samples were allowed to equilibrate at room temperature for 5 minutes. The quantitative absorbance of the separated upper hexane phase layer was recorded at 503 nm against a pure hexane blank phase.

Estimation of Total Phenolic Content (TPC)

The concentration of total structural polyphenols within the extract was determined using the Folin-Ciocalteu assay adapted from Donald and Johns (2002). A 0.5 mL portion of the extract solution was treated with 0.1 mL of 0.5 N Folin-Ciocalteu reagent and held at room temperature for 15 minutes. Subsequently, 2.5 mL of a 20% sodium carbonate (Na_2CO_3) neutralizing solution was added, and the total reaction volume was brought to 10 mL using distilled water. The sample tubes were incubated in complete darkness for 30 minutes at room temperature, after which the absorbance was recorded at 760 nm. Standard calibration linear regressions were generated using standard Gallic acid lines (1.0 to 12.0 mg/mL), and the total phenolic concentrations were expressed as milligrams of Gallic Acid Equivalents per gram of total dry weight base (mg GAE/g).

Evaluation of DPPH Radical Scavenging Antioxidant Activity

The free-radical scavenging capacity of the extract was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) protocol derived from Soma and Genitha (2014). A working DPPH assay solution (0.048 mg/mL) was prepared by diluting a stock methanolic DPPH concentrate. A 500 µL sample of the extract was combined with 500 µL of the working DPPH solution, mixed thoroughly via vortexing, and incubated in darkness for 30 minutes at room temperature.

The absorbance of the solution was measured at 515 nm against a pure methanol blank using a spectrophotometer. The percentage of radical inhibition was calculated using the following formula:

$$\text{Total Antioxidant Activity (\%)} = [(A_{(c)} - A_{(s)}) / A_{(c)}] \times 100$$

Where:

- $A_{(c)}$ = absorbance of the DPPH control solution (without extract)
- $A_{(s)}$ = absorbance of the DPPH solution containing the papaya extract

Statistical Controls

Every experimental combination was performed in triplicate (n = 3). The resulting data was evaluated using analysis of variance (ANOVA) and critical difference (CD) metrics via MS Excel software (2019) to verify statistical significance at a 5% significance level ($\alpha = 0.05$).

Results and Discussion

Kinetic Evaluation of Process Parameters on Extract Yield

The processing performance changes in gravimetric mass recovery across the different extraction temperatures and acoustic contact times are summarized in Table 1.

Table 1: Gravimetric Yield Extraction Percentage (%) under Diverse UAE Conditions

Extraction Time (min)	45°C Yield (%)	55°C Yield (%)	65°C Yield (%)
15	72.50 ± 0.04	82.50 ± 0.03	72.50 ± 0.05
20	75.00 ± 0.03	82.90 ± 0.02	75.00 ± 0.04
25	80.00 ± 0.02	87.50 ± 0.02 (Optimal)	77.50 ± 0.03
30	79.50 ± 0.05	85.00 ± 0.04	82.50 ± 0.02

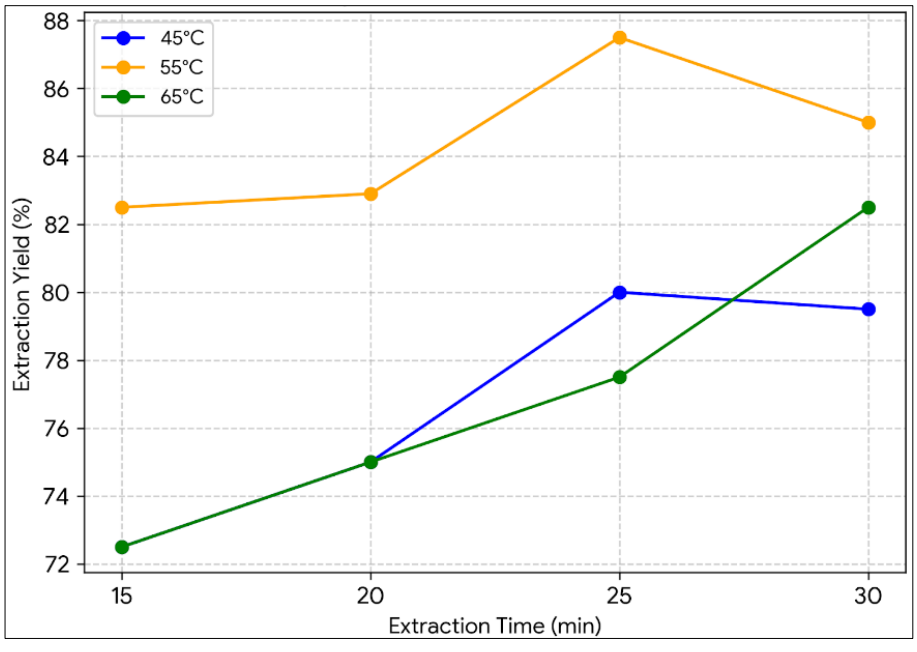

Fig 1: Effect of Temperature and time on Extraction Yield

The empirical results demonstrate that extraction yield performance depends on both the processing temperature and the duration of acoustic contact. Increasing the extraction temperature from 45°C to 55°C significantly increased total mass recovery. This improvement is attributed to changes in the physical properties of the system: higher temperatures reduce solvent viscosity, increase solute solubility, and improve cell wall permeability, allowing the aqueous ethanol to thoroughly penetrate the dried papaya powder matrix. Furthermore, acoustic cavitation intensity is maximized near 55°C, accelerating cellular wall rupture and increasing surface-area contact between the solid and liquid phases.

However, increasing the extraction temperature to 65°C caused a decrease in yield performance during shorter processing runs. This decline occurs because higher processing temperatures increase solvent vapor pressure, which introduces excess vapor into the cavitation bubbles. This vapor dampens their implosion intensity during acoustic cycles, reducing the mechanical shear forces required to break down cell walls.

Time kinetics followed a similar trend, with peak yield performance achieved at 25 minutes across all evaluated temperatures. Extending the processing run to 30 minutes at 55°C resulted in a measurable loss of recovery efficiency. This downward trend is likely due to the structural collapse of cavitation bubble dynamics and the progressive thermal degradation of highly unsaturated lipophilic pigments under extended acoustic shear stress. These observations align with the findings of Nazia *et al*. (2016), who reported that solvent characteristics, raw matrix structure, and temperature-time combinations significantly affect mass transfer kinetics during botanical extraction.

Phytochemical Fingerprinting of the Optimized Extract

The papaya powder extract generated under optimal operating conditions (55°C for 25 minutes) was analyzed to establish its bioactive composition prior to functional food formulation.

Table 2: Compositional Characterization of the Optimized Ripe Papaya Powder Extract

Parameter	Value
β-Carotene	85.315 ± 0.02 mg/100 g
Lycopene	55.568 ± 0.02 mg/100 g
Total Phenolic Content	205.700 ± 0.03 mg/100 g
DPPH Activity	25.000 ± 0.02 %
Extraction Yield	87.500 ± 0.02 %

*Values represent the average of three individual test replications (n = 3).

The optimized extract exhibited strong functional properties, yielding a β-carotene content of 85.315 ± 0.02 mg/100g and a lycopene content of 55.568 ± 0.02 mg/100g. These levels confirm that UAE is highly effective at recovering lipophilic carotenoids from pre-dried fruit matrices. The total phenolic content reached 205.70 ± 0.03 mg/100g, while the DPPH radical scavenging capacity was measured at 25.00 ± 0.02%. This high level of antioxidant activity is driven by the synergistic interactions between the carotenoid double-bond systems and polyphenolic molecules, which neutralize free radicals by donating hydrogen atoms to stabilize oxidative chain reactions. These values align with the findings of Veda *et al*. (2014) and Ali *et al*. (2011) [1], who reported similar ranges for total phenolic content and antioxidant retention in clean papaya matrices, confirming that optimized ultrasound processing successfully protects the structural stability of these heat-sensitive molecules.

Conclusion

The present work investigated the effectiveness of ultrasound-assisted extraction in recovering bioactive carotenoids and antioxidants from ripe papaya powder and identified the optimum extraction conditions. Processing at a temperature of 55°C for an acoustic contact time of 25 minutes using a 1:50 solid-to-solvent ratio of 60% aqueous ethanol maximized mass transfer kinetics, achieving a total gravimetric yield of 87.50%. The resulting pure extract retained high concentrations of β-carotene (85.315 mg/100g) and lycopene (55.568 mg/100g), while preserving a strong total phenolic profile and antioxidant capacity. These results demonstrate that this optimized papaya powder extract can serve as a functional, bioactive ingredient, offering a viable, health-promoting alternative to synthetic colorants and stabilizers in clean-label food and beverage applications.

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